Using Mechanism of Action and Structural Similarity Information to Reduce the Default Uncertainty for Subchronic to Chronic Exposure for the Bromobenzene Reference Dose (RfD) and Reference Concentration (RfC).

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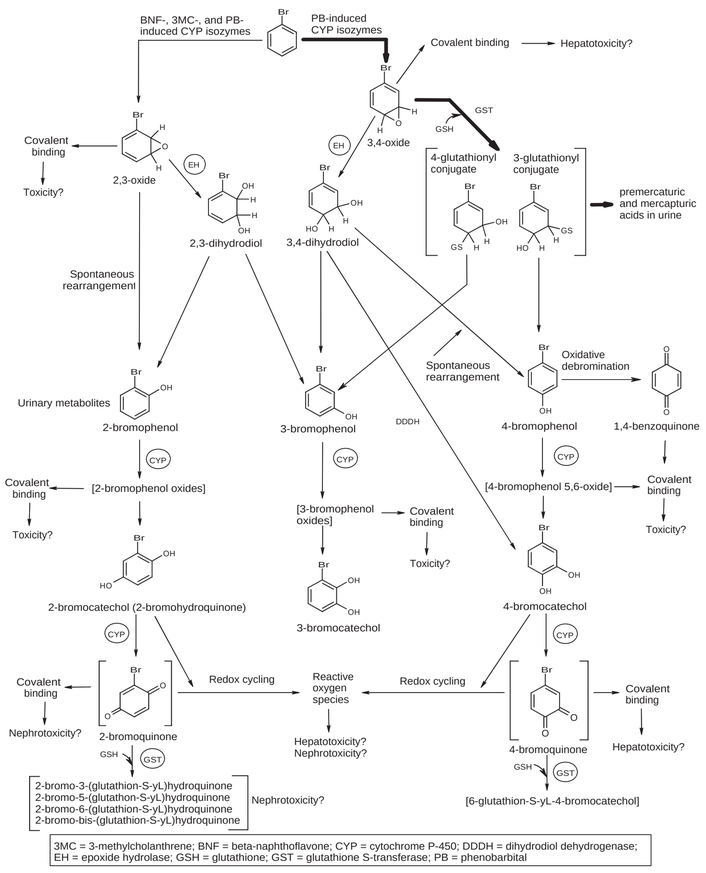
Due to the limited data available for risk assessment for some chemicals uncertainty can be quite high. Bromobenzene is an example of a chemical for which no chronic studies are available. This poster explores the possibilities of reducing subchronic to chronic uncertainty based on what is known about bromobenzene's mechanism of action and its structural similarities to chlorobenzene. Bromobenzene appears on the Contaminant Candidate List (CCL) but is not on the Integrated Risk Information System (IRIS). EPA is in the process of developing a Toxicological Review for bromobenzene for IRIS. No data were located regarding the toxicity of bromobenzene in humans. Animal studies identify the liver as the most sensitive target of oral and inhalation exposure to bromobenzene. Numerous mechanistic studies in animals demonstrate that hepatotoxicity is associated with the metabolism of the parent compound by one or more reactive metabolites. Repeated dose studies have shown that the liver appears to develop a tolerance to bromobenzene insult. By the tenth dose of 315 mg/kg of bromobenzene, glutathione depletion was less, serum liver enzymes were no longer increased and liver lesions were not seen. Bromobenzene and chlorobenzene exhibit striking similarities in structure, toxicokinetic properties and critical targets of toxicity (liver) in rats and mice. Metabolic schemes for bromobenzene and chlorobenzene include cytochrome catalyzed epoxidation to reactive epoxide intermediates and subsequent formation of corresponding metabolites. No chronic oral or inhalation studies are available for bromobenzene. However, a chronic study is available for chlorobenzene. Dose-response relationships for liver effects from subchronic and chronic exposure to chlorobenzene appear to be similar, which suggest the development of some degree of tolerance to chlorobenzene during chronic exposure. It is reasonable to expect such similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene. Therefore, a factor of 3 rather than the default of 10 may be appropriate to extrapolate from subchronic to chronic exposure to bromobenzene. (This presentation does not necessarily reflect the views and policies of the U.S. EPA.)



Proposed Metabolic Scheme for Bromobenzene in Mammals
(Adapted from Lau and Monks, 1988; Lertratanangkoon et al., 1993)

Bromobenzene Hepatotoxicity

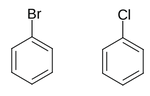
- Hepatotoxicity is associated with the metabolism of the parent compound and cytotoxicity results from modifications of hepatocellular macromolecules by one or more reactive metabolites primarily via the 3-4-oxide pathway.
- Glutathione conjugation provides a protective mechanism for acute hepatotoxicity.
- Hepatotoxicity has been demonstrated in oral subchronic studies in rats and mice (NTP, 1985a,b) by significant increases in serum liver enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Sorbitol dehydrogenase (SDH), and increased liver lesions.

Evidence for Tolerance to Bromobezene Hepatotoxicity

- Repeated dose experiments (Kluwe et al., 1984) show that single oral doses of 315 mg/kg-day administered to male rats daily for 10 days resulted in glutathione depletion, liver lesions, and increased ALT and SDH. Following the 10th dose glutathione depletion was less pronounced, ALT and SDH were no longer increased and liver lesions were not seen.
- NTP (1985a,b) assessed serum ALT and AST and SDH levels in male and female rats. These enzyme levels were significantly (approximately 30-100 fold) increased after the first treatment. On the third day of treatment these enzymes remained elevated but the magnitude decreased to approximately 2-6 fold above controls levels. At day 94, these serum enzymes were not significantly elevated.

Similarities Between Bromobenzene and Chlorobenzene

- **Structure**



- **Absorption**
  Widely distributed with highest levels in adipose tissues
- **Metabolism**
  Cytochrome P-450 catalzyed epoxidation to reactive epoxide intermediates, and subsequent formation of:
  - di-hydrodiol derivatives
  - phenols
  - glutathione conjugates
  - catechols
  - quinones
- **Elimination**
  Urinary excretion of derived metabolites.

Similarities in Bromobenzene and Chlorobenzene Subchronic Oral Toxicity

Studies in male and female rats and mice identified the liver and kidney as the most sensitive targets of toxicity.

- For bromobenzene, nephrotoxicity has been observed in animals following exposure at higher doses than the lowest hepatotoxic dose.
- In general, liver and kidney effects produced by bromobenzene occur at oral doses that are within the same order of magnitude as those produced by chlorobenzene.

Comparison of adverse effect levels for bromobenzene- and chlorobenzene-induced histopathologic liver and kidney lesions in the 90-day (13 week) oral gavage studies conducted by NTP (1985a,b,e) using male and female F344/N rats and B6C3F1 mice

Effect level (mg/kg -day)	Liver		Kidney	
	NOAEL	LOAEL	NOAEL	LOAEL
Male rats				
Bromobenzene	100	200	400	600
Chlorobenzene	500	750	750	not determined
Female rats				
Bromobenzene	200	400	400	600
Chlorobenzene	500	750	500	750
Male mice				
Bromobenzene	100	200	400	600
Chlorobenzene	125	250	125	250
Female mice				
Bromobenzene	100	200	600	not determined
Chlorobenzene	125	250	125	250

Data Available for Structurally Similar Chlorobenzene

Reproductive Toxicity

Two generation study in rats exposed to vapor concentrations (0, 50, 150, 450 ppm daily, 6 hours/day for 10-11 weeks prior to mating, throughout mating gestation and lactation (Nair et al. 1987)

- Increased incidences of liver and kidney lesions in F0 and F1 male rats exposed at >150ppm.
- At 450ppm (highest exposure level tested) no clear sign of reproductive toxicity in either generation.

Developmental Toxicity

- No developmental effects in fetuses of pregnant rats exposed to vapor concentrations as high as 590 ppm 6 hours/day on gestation days 6-15 for 13 weeks (John et al., (1984).
- Charles River albino rat dams administered oral dose levels of 100 or 300 mg/kg-day on gestation days 6-15 produced no developmental toxicity (IBT, 1977), however it is uncertain whether repeated oral doses of chlorobenzene as high as those known to induce histopathologic liver lesions in rats (750 mg/kg-day) might cause developmental effects.

Data Available for Bromobenzene

- No chronic oral or inhalation animal studies are available for bromobenzene
- Subchronic oral gavage studies (NTP,1985a,b) in rats and mice did not reveal evidence of significant treatment related effects on reproductive organs at dose levels that are hepatotoxic.

Evidence for Tolerance to Chlorobenzene Toxicity

- A 2-year toxicity and carcinogenicity study (NTP, 1985e) in male and female F344/n rats and B6C3F1 mice (50/sex/species) administered doses of 0, 60, or 120 mg/kg-day (0, 30, or 60 mg/kg-day for male mice), 5 days/week for 2 years by oral gavage showed no evidence of treatment-related increased incidences of nonneoplastic liver lesions in female rats or male or female mice.
- The 2-year study identified a free-standing no-observed-adverse-effect level (NOAEL) of 120 mg/kg-day in female rats and equivocal evidence of a lowest-observed-adverse-effect level (LOAEL) of 120 mg/kg-day for hepatocellular necrosis in male rats. In male and female mice, free-standing NOAELs were 60 and 120 mg/kg-day, respectively, for nonneoplastic liver effects.
- In a similarly-designed subchronic (90-day) oral toxicity study in mice, a NOAEL of 125 mg/kg-day was identified in both males and females; the LOAEL was 250 for chlorobenzene-induced liver lesions (NTP, 1985e).

Conclusions and Risk Assessment Implications

- Bromobenzene and chlorobenzene exhibit striking similarities in structure, toxicokinetic properties, and critical target of toxicity (liver) in rats and mice.
- Subchronic oral studies in both male and female rats and mice identify the liver as a critical target of bromobenzene toxicity.
- A chronic oral toxicity study available for chlorobenzene demonstrates some tolerance to chlorobenzene during chronic exposure (i.e., dose-response relationships for liver effects from subchronic and chronic exposure appear to be similar). It is reasonable to expect such similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene as well because mechanistic studies have demonstrated the development of some degree of tolerance upon repeated exposure to bromobenzene (Kluwe et al., 1984).
- Reproductive and developmental endpoints may not be sensitive endpoints for chlorobenzene or bromobenzene. A reduction in the database uncertainty factor for bromobenzene was considered based on what is known about structurally similar chlorobenzene. However, since bromobenzene lacks developmental toxicity studies, multi-generation reproductive studies, neurotoxicity studies and and chlorobenzene lacks a developmental study in a second animal species, the database uncertainty factor for bromobenzene RfD and RfC are considered to be a full factor of 10 .
- An uncertainty factor of 3 (rather than the default value of 10) is being considered to account for extrapolation from subchronic to chronic exposure to bromobenzene for determination of an RfD and RfC based on evidence that tolerance to bromobenzene induced hepatoxicity develops after repeated exposure.

References

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